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(54) Title: METHOD AND COMPOSITIONS FOR CELLULAR REPROGRAMMING

(57) Abstract

The present invention provides methods and compositions useful in treating certain diseases herein termed "Aberrant Programming Diseases", including cancer and AIDS. According to one aspect of the invention, there is provided a method for treating an individual having an Aberrant Programming Disease comprising administering to said individual an effective amount of a composition selected from the group consisting of an expression vector, a double stranded oligodeoxynucleotide, and an antisense oligodeoxynucleotide; said composition capable of regulating expression of a transcriptional regulator, said transcriptional regulator being expressed by the Aberrant Programming cells and further characterized by exhibiting a therapeutically useful change in said cell behavior in the Reprogramming Test. In a separate embodiment new antisense oligodeoxynucleotides are provided.

METHOD AND COMPOSITIONS FOR CELLULAR REPROGRAMMING

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to methods and
5   compositions useful in treating disorders in which the
direct cause of the clinical disorder is the expression
in the primary diseased cells of a differentiation
program that does not normally exist. Such disorders
are hereinafter referred to as Aberrant Programming (AP)
10   Diseases. The invention also relates to method and
compositions useful in therapeutically reprogramming
normal cells.

As will be discussed more fully hereinafter, the AP
diseases of this invention constitute a new disease
15   classification and there is presented a novel molecular
model of pathogenesis for these diseases. According to
the molecular model of this invention, the basic disease
causing entity in the AP diseases is a specific type of
relational alteration among certain cellular components
20   involved in program control. It is unlike any
previously described molecular pathogenic mechanism.
This model defines the nature of the therapy for these
diseases, limits the potential set of therapeutically
useful targets to a relatively small number of genes and
25   leads to the unobvious conclusion that this includes the
manipulation of certain "normal" genes is an appropriate
approach for the treatment of AP diseases, thus, leading
to a unique approach to therapy for the AP diseases of
this invention. This model makes the selection of

targets for proposed therapy straightforward and accessible to anyone skilled in the art.

A preferred embodiment relates to the reprogramming of cell behavior through the manipulation of transcriptional regulators (TRs). The invention includes systemic treatment and compositions for such treatment, as well as _in_ vitro manipulation of cells prior to transplantation of such cells with the host under treatment.

10   DESCRIPTION OF THE RELATED ART

Very recent studies involving the use of antisense oligonucleotides for treatment of cancer have been reviewed by Stein and Cohen, Cancer Res. 48:2659 (1988).
15   Several types of antisense molecules have been screened for their ability to inhibit the synthesis of particular proteins using both intact cells and in vitro systems for protein synthesis (See Ld. and Paoletti, Anti-Cancer Drug Design 2:325, 1988). For example, agents with
20   specificity for RNA transcribed from the myc gene have been reported to inhibit the proliferation of the human AML line HL60 (Wickstrom, et al., Proc. Natl. Acad. Sci. USA 85:1028 (1988) and normal T lymphocytes (Heikkila, et al., Nature 328:445 (1987), and oligodeoxynucleotides
25   complementary to cyclin mRNA have been reported to suppress the division of 3T3 cells (Jaskulski, et al. 1988).

More recently, it has been found that in the treatment of cancer with ODNs against myb, the
30   proliferation of leukemic cells was inhibited with an accompanying lower degree of inhibition against normal cells. (Calabretta et al, PNAS, 88, 2351, 1991.) Also,

it has been shown that transient inhibition in a leukemia cell line resulted with an ODN against myc; however, unfortunately, a comparable inhibition against normal cells occurred (Zon et al patent). This patent

5   also discloses inhibition of HIV replication using ODNs targeted to viral genes. Belenska et al (Science, 250, 997, 1990) have proposed the use of double stranded ODNs, binding to TR ligands as potential therapeutic agents for disease causing genes. They give blocking

10   of NF-kB binding to HIV enhancer as an example. The use of retroviral vectors carrying antisense oncogenes for the treatment of cancer is known.

The fundamental problem with the foregoing part is that it is based on the notion that the expression of

15   specific molecular abnormalities (altered regulation or mutation of endogenous genes or expression of exogenous genes) in the disease cells of these patients directly cause the clinical pathological features of the AP disease. It follows from such thinking that the

20   therapeutic strategies should be, directed to attacking these molecular abnormalities.

In the case of cancer, contemplated therapy involving antisense expression vector ODNs have been directed to oncogenes in accordance with the

25   oncogene/anti-oncogene cancer model, or to growth factors expressed by cancer cells in accordance with the autocrine model. In the case of AIDS therapeutic strategies involving such agents being developed are directed toward blocking HIV expression and/or

30   infection. There are no counterpart causal agents identified to the other AP diseases. Hence the therapeutic approaches under development are more empirical.

According to the AP disease model the fundamental
pathology causing the clinical pathological features of
these disorders is both relational and dynamic. In
stark contrast to the prior art, the therapy of the
5   present invention involves manipulation of patterns of
TR expression. The invention provides an entirely new
approach to the treatment of said selected diseases and
provides a rational, empirical basis for the design of
novel agents. The therapeutic reprogramming of normal
10   tissue involving ODNs is unprecedented.

SUMMARY OF THE INVENTION

In accordance with this invention, there is provided
a method for reprogramming cell behavior to achieve
15   therapeutic effects through manipulating patterns of TR
expression.

Also provided is a method for treating an individual
having an AP disease comprising administering to said
individual an effective amount of a composition selected
20   from the group consisting of an expression vector, a
double stranded ODN, and an antisense ODN. Said
composition must be capable of regulating expression of
a TR. Said TR is expressed by the AP cells and further
characterized by the fact that it exhibits a
25   therapeutically useful change in said cell behavior in
the Reprogramming Test of this invention (hereinafter
more fully described). It is noted that when the AP
disease is AIDS, said TR is not encoded by HIV. In the
case of cancer, said TR is a Traitor Gene of this
30   invention (more fully discussed hereinafter) and,
preferably, excludes oncogenes, e.g. fos, myc, myb, rel,
jun (in an altered form).

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Another embodiment of this invention is a method for treating an individual having a clinical disorder comprising administering to said individual an effective amount of a composition selected from the group consisting of a double stranded ODN and an antisense ODN. The composition is capable of regulating expression of a TR. The TR is expressed by therapeutically relevant cells and is further characterized by exhibition of a therapeutically useful change in said cell behavior in the Reprogramming Test of this invention.

The invention revealed here primarily embodies a new type of therapy based on reprogramming cellular behavior. Collateral inventions, however, also follow including: (1) the diagnosis and/or staging of aberrant programming diseases by assaying for the expression of particular transcriptional regulators and their variants in diseased cells; and, (2) for any given aberrant program disease, the use of test agents _in_ _vitro_ for determining the optimum agent(s) for treating any particular patient.

Thus, there is provided a method for diagnosing or staging an AP disease comprising identifying the relevant subset of TRs expressed by AP cells from an AP patient. A method for selecting the most efficacious treatment regimen for an AP disease forms another embodiment. This embodiment comprises identifying the relevant subset of TRs expressed by AP cells from an AP patient. These embodiments are described more fully hereinafter.

In addition, the invention provides a method for treating therapeutically relevant cells from an individual having a clinical disorder prior to transplantation of the cells back into the individual
5   (autologous transplant) embodiment. This embodiment comprises the steps of:

a) obtaining therapeutically relevant cells from the individual and

b) exposing the therapeutically relevant cells to a
10 reprogramming amount of an ODN having a sequence complementary to a sequence of RNA transcribed from a TR regulated gene or double stranded ODN ligand of a transcriptional regulator present in the TR cells. In a preferred embodiment the cells are taken from prenatal
15 tissue or from a different donor than the individual under treatment (allogeneic transplant).

Selection of the most efficacious treatment regimen for an AP disease forms another embodiment of this invention. This method involves removing and culturing
20 AP disease cells from an AP disease patient with an antisense ODN specific to a TR from the relevant subset of TRs expressed by AP cells from an AP patient or a double stranded ODN to the DNA binding domain of such TR to determine optimal treatment.

25 In carrying out the methods of treating AP diseases of this invention it is critical to select the proper targets. Hence, an important embodiment of this invention is a method for the selection of a target for the treatment of an AP disease comprising (i)
30 determining the subset of transcriptional regulators and their direct modifiers expressed by the aberrantly

programmed tissue, the corresponding normal tissue, or
the constitutively self-renewing normal tissue or,
alternatively, making a similar determination for any
other normal tissue that is to be therapeutically
5   manipulated in accordance with this invention; (ii)
adding or subtracting expression of transcriptional
regulator(s), or their direct modifiers, from cells to
be therapeutically reprogrammed and the appropriate
control tissue; (iii) scoring effect on cellular
10   programming and selecting potential therapeutic agents
according to the Reprogramming Test; (iv) testing effect
of addition or subtraction of the function of particular
transcriptional regulators, using the agents selected,
(in an animal model system if the therapeutic agents are
15   for systemic use), and (v) reducing or eliminating any
undesirable side effects that might be produced by the
potential therapeutic agents. This embodiment is
described in detail hereinafter.

Exploiting specific cell type differences in target
20   RNA for selecting differentially available sites for ODN
binding forms another embodiment of this invention.
This embodiment comprises a method for cell type
dependant targeting of specific RNA transcripts
comprising selecting an ODN capable of binding to and
25   leading to the destruction of said RNA in the tissue to
be therapeutically manipulated, but not in tissue where
side effects are produced by destruction of said RNA.
Exemplary is the use of an antisense ODN directed to
cyclooxygenase RNA that selectively binds to and
30   destroys said RNA in hematopoietic tissue while avoiding
said RNA in gastrointestinal tissue.

All of the foregoing embodiments involve reprogramming of cell behavior to achieve therapeutic effects through manipulating patterns of TR expression.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

5   AP Disease Model and List

Definition of a "cellular program"

The coordinated appearance in cells of a cell type restricted pattern of gene expression over time that provides for a particular phenotype
10   and as a result for the determination of the range of possible cellular responses to exogenous stimuli.

The fundamental program can be thought of as a differentiation program which in turn controls
15   the subprogram responses of the cell to environmental and other exogenous cues where the subprograms include cellular viability (apoptosis) and proliferation.

Definition of an "Aberrant Programming Disease"

20   One in which the direct cause of the clinical disorder is the expression in the primary diseased cells of a differentiation program that does not normally exist. That is, there is an expression of normal genes that provide for
25   particular differentiated phenotype in abnormal combinations. The result is that these diseased cells become capable of expressing pathogenic

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behaviors involving cellular differentiation, viability and proliferation. These attributes of the primary diseased cells can also induce pathologic change, in their tissue environment.

5.   The term "direct cause" with respect to pathogenesis is to be distinguished from "risk factors." Typically an AP Disease will be associated with numerous risk factors that in various combinations appear to "cause" the appearance of the disease. In fact, however, they cause the changes in the pattern of transcription regulator (TR) expression and chromatin domain availability which in turn causes the disease. This is important because programs can evolve and can become independent of any risk factors involved in their induction. Risk factors include mutagenic events, viruses, chromosomal abnormalities, genetic inheritance, and diet.

Aberrant programming disorders can be manifested as either a hyperplastic or a hypoplastic (degenerative) disease or a combination of both.

Examples of diseases where the aberrant program phenotype is expressed:

Cancer

Myeloproliferative Diseases
- polycythemia vera
- agnogenic myeloid metaplasia
- essential thrombocytosis

Myelodysplasias

- refractory anemia
- refractory anemia with ringed sideroblasts
- refractory anemia with excess blasts
- refractory anemia with excess blasts in transition

Atherosclerosis

AIDS-related complex

AIDS

Molecular Model

According to the molecular model set forth herein, the basic disease causing entity in the AP diseases is a specific type of relational alteration among certain cellular components involved in program control. It is unlike any previously described molecular pathogenic mechanism. This model defines the nature of a novel therapy for these diseases, limits the potential set of therapeutically useful targets to a relatively small number of genes and leads to the unobvious conclusion that the manipulation of certain "normal" genes is an appropriate approach for the treatment of AP diseases, in this way the model makes the reduction to practice of the proposed therapy straightforward and accessible to anyone skilled in the art.

Specifically, the essential molecular pathology in the AP diseases consists of changes in the interdependent patterns of TR expression and/or chromatin domain availability for transcription: In turn, these relational alterations provide for the expression of abnormal cellular programs involving

cellular differentiation which are pathogenic. Particular TR or certain molecules involved with the control of domain status may be structurally abnormal. However, these are not necessarily useful targets for therapeutic intervention.

Tenets of the model relevant to the development of specific therapy:

A) Those true of both normal and aberrant programming:

1) The pattern of domain availability determines the possible range of genes that can be expressed in the cell and, therefore, limits the range of cellular programs that can be expressed.

2) The pattern of TR expression is the molecular equivalent of a programming code. By analogy with language particular combinations of TR (letters) working as a unit (words) regulate the expression of sets of genes in a coordinate fashion while the complete set of TR combinations used in any given cell (sentence) determines which of the possible phenotypes the cell will expressed, and therefore the overall character of the cell's differentiation program (see Table I for more details where cancer is used as an example).

3) Only a subset of the total number of TRs involved in the control of cellular differentiation for the total organism are

expressed in any given cell type and they are few in number.

4)   Similar effects on particular patterns of gene expression (programming) can be achieved by more than one specific combination of TR (synonyms).

5)   The specific functional consequences of a particular TR's being expressed is context-dependent.   That is, its effects on cellular programming depends both on which other TR it combines to regulate a particular set of genes (what words it appears in) and on the total set of different TR combination expressed by the cell (the sentence).

B)   True of AP cells but not normal cells:

1)   The combinations of TR seen in AP cells is different from that seen in any normal cell (the sentence is not expressed by any normal cell).

2)   The specific functional consequences of any given particular TR being expressed in an AP cell, therefore, will be different from the consequences seen in a normal cell.

3)   AP cells, therefore, express a cellular differentiation program that is different from any normal differentiation program. As a result AP cells express pathogenic behaviors resulting from their altered differentiation, viability and proliferation characteristics.

4)   Hence, equivalent manipulations of the
expression of a given TR in normal cells vs.
aberrantly   programmed   cells   can   produce
differential effects on cellular behavior. This
can form the basis of therapeutic intervention.

5)   The subset of TRs expressed by any AP cell
is expected to include TR not expressed by the
corresponding normal cells and/or conversely.
These TRs within the AP cells will be normal TRs
ectopically expressed or modified (alternate
splicing promoter use or post-translational
modification) or mutated to a TR with altered
binding properties.

Nature of Targets

TRs   are   the   primary   targets   for   therapeutic
manipulations based on the model. They may be
manipulated directly or indirectly through molecules
such as tyrosine kinase, that can effectively change a
TR of one type to another through structural alterations
such as phosphorylation.

Nature of Therapeutic Intervention

The basis of the novel therapy is to differentially
change the pattern of gene expression in AP cells by
altering the pattern of TR expression. The model states
that   the   specific   functional   consequences   of   the
expression of any given TR is context-dependent. It
therefore follows that the same TR present in both
normal and AP cells can be manipulated in the same way
and a different impact on cellular behavior obtained.

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A TR expressed only by the AP cells, however, also may
be targeted. The end result is that the pattern of gene
expression in the AP cells lose at least a substantial
portion of their disease-producing activity. This can
5   be manifested in numerous possible ways including death
of the AP cells, a change in their differentiation
status with a concomitant change in the production of
disease-producing factors or to a loss of proliferative
potential.

10   The number of transcriptional regulators that will
have to be manipulated in any given cell type will be
very small. There are estimated to be 30,000 to 100,000
genes in the human genome distributed over 3×10^9 bp of
DNA. In any given cell type approximately 10,000 genes
15   can be shown to be expressed. Greater than 90% of these
are expressed by many cell types and the large majority
of these are referred to as "housekeeping genes."

Typically, the number of genes that can be shown to
be differentially expressed in any given cell type
20   account for only a few hundred. It is these genes that
make the difference between liver cells and brain cells,
for example. The large majority of these are directly
involved in carrying out the functions that characterize
the cell type. Liver cells, for example, express a wide
25   range of enzymes that are involved in ridding the body
of many types of chemicals. The genes of interest for
the purposes of this patent are the small subset of
genes coding for molecules involved in the differential
regulation of cell type specific genes. In particular,
30   transcriptional regulators and their direct modulators.
The latter includes, for example, certain tyrosine
kinases, that can modify a particular transcriptional

regulator and, in effect, change it to a functionally
different transcriptional regulator. (Berk Biochem
Biophys. Actn. 1009, 103, 1989) For the purposes of
this invention transcriptional regulators are defined
5   as molecules that bind to specific DNA sequences
variably expressed by different genes and/or to other
transcriptional regulators at least one of which must
bind to specific DNA sequences. As a result they
control the levels of gene expressions by means of
10 modulating RNA polymerase activity. The transcriptional
regulators may be of either endogenous or exogenous
origin. They may either be normal or be mutated.

The ability of transcriptional regulators to
variably interact with each other provides the basis for
15 a combinational regulatory system. This allows a very
small number of transcriptional regulators to control
the expression of a large number of genes in various
patterns. Particular sets of genes being controlled at
any given time by a certain subset of the
20 transcriptional regulators being expressed by the cell.
Each transcriptional regulator subset, therefore, is a
programming code or an instruction or a "word" that
directs the expression of a particular gene set. The
entire pattern of gene expression being expressed by a
25 given cell type can be thought of as a sentence, since
only certain words can appear together.

A general role for combinatorial regulation being
involved in eukaryotic gene expression has been
previously postulated by several investigators.
30 (Scherrer, and Marcand J. Cell Phys 72, 181, 1968;
Sherrer Adv. Esp. Med. Biol. 44, 169, 1924; Gierer Cold
Spring Harbor Symp Quant Biol 38; 951, 1973;
Stubblefield J. Theor Biol 118, 129, 1986, Bodnar J.

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Theor Biol <u>132</u>, 479, 1988) Lin and Riggs (Cell <u>4</u>, 107, 1975), demonstrated using biophysical arguments the impossibility of having a separate regulator for every gene in a eukaryotic cell. Combinatorial regulation

5   models of eukaryotic gene expression generally postulate multiple levels of regulation in addition to transcription. In principle, these models show how theoretically 100,000 genes could be selectively controlled by as few as 50 regulatory molecules only a

10   small subset of which would operate at the level of what is defined here as transcriptional regulators. Bodnar J. Theor. Biol. <u>132</u>, 479, 1988.

The actual number of human transcriptional regulators are estimated to number on the order of somewhere in

15   excess of 100. (Table II lists those that have been described in the literature.) Many, however, are known to be expressed only in certain cell types. Since just a few hundred genes determine the differences between particular differentiated cell types and the large

20   majority of these determine the particular functional features of the cell, only a very small number of these can be regulator gene products.

It follows, therefore, that the number of regulators that must be manipulated to achieve the effects

25   stipulated by this invention for any given application is small and can be managed with comparatively modest effort. It also follows from the notion of combinatorial regulation that not all the transcriptional regulators expressed by a given cell

30   type need to be known before this invention can be practiced.

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The present inventor has found that antisense p53 oligonucleotides can inhibit the proliferation, including the blocking of stem cell self-renewal, and ultimately kill primary human leukemic blasts while not producing similar effects on fresh normal bone marrow cells. This unobvious result indicates that the interactive mechanisms for detecting, interpreting and responding to environmental informational molecules involved in regulating cell differentiation and proliferation and viability in AP cells are so altered from normal in terms of their dynamic interactions (involving signal transduction and interpretation) that the inhibition of a single gene or set of genes coding for proteins involved in this process by antisense oligonucleotides is sufficient to change the impact of the informational molecules so a change in cellular programming such as cellular death or growth inhibition program can be selectively instituted in AP cells. The term "traitor genes" is used herein to describe those genes in AP cells that may be suitable for targeting for inhibition with antisense molecules in accordance with the present invention Suitable target or traitor genes may themselves either be functionally abnormal or be normal but function to maintain the pathological phenotype AP cells as part of an abnormal pattern of gene expression. Such treatment results in differential programming of AP cells, but not their normal counterparts over a selected dose range. In the preferred embodiment the Traitor Genes to be targeted are TRs.

The concentration of oligonucleotide to be used may vary, depending upon a number of factors, including the type of cancerous cells present in the marrow, the type, and the specificity of the particular antisense

oligonucleotide(s) selected, and the relative toxicity of the oligonucleotide for normal cells. Although the present inventor has observed significant AP cell programming at oligonucleotide concentrations in extracellular fluid as low as 1 nanomolar, optimal inhibition was observed at concentrations of at least 10 nanomolar in the model system described below. The upper limit of the dosage range is dictated by toxicity and therapeutic efficacy, and, generally will not exceed 5 micromolar. With the aid of the techniques set forth in the present disclosure, those of skill in the art should be able to determine the optimal concentration to be used in a given case.

"Hardware" for Reduction to Practice

Using established techniques, assays and agents, the following capabilities can be readily acquired. These can be used by anyone skilled in the art to reduce the primary and collateral inventions to practice.

1)   Assays for transcriptional regulators and their direct modifiers.

Preferred assays: RNA _in situ_ hybridization (Lum Biotech. 4, 32, 1986) or PCR (Block, Biochem 30, 2735, 1991) or metabolic labelling (Ausubel et al (eds.) Current Protocols in Molecular Biology, John Wiley NY, 1989 (updated semiannually)) for detecting expression at the protein level.

Purposes:

To establish the subset of the known
transcriptional regulators or their direct
modifiers that are expressed by a particular
cell type. This will serve the following

5   functions:

a) the determination of the subset of
transcriptional regulators, or their direct
modifiers, that are targets to be manipulated in
the reduction to practice;

10   b) the evaluation of the effectiveness of
potential therapeutic agents in adding or
subtracting the expression of a particular
transcriptional regulator or its direct modifier
cells;

15   c) the diagnosis and/or staging of a
particular aberrant program disease;

d) the determination of the optimum
therapeutic agent(s) in clinical practice, when
there are more than one option for a given

20   disease.

2) Agents for adding or subtracting the expression
of particular transcriptional regulators or
their direct modifiers in cells to be
therapeutically manipulated.

25   a) Antisense oligonucleotides (Zon,
Pharmaceut. Res., $\underline{5}$, 539, 1988).

These agents can be used to subtract the
expression of particular genes from cells.

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Design of "test" antisense oligonucleotides

i) Using a computer program such as "Oligo" (Rychik and Rhoads, Nucl. Acids Res., 17, 8543, 1989) select a set of antisense oligonucleotides that bind to the RNA target of choice that have the following characteristics: (1) length between 10 and 35 bases with 20 being generally used; (2) negligible self-interaction (self-dimers and hair pins) under physiologic conditions; (3) melting temperature ≥ 40°C under physiological conditions; and (4) no more than 40% of the oligonucleotide being a run of guanines or cytosines);

ii) Using a reference such as Genbank ensure that the antisense oligonucleotide has ≤ 85% homology with the RNA transcripts of other genes. An exception to this is where an antisense oligonucleotide is selected on the basis of its ability to bind to more than one member of a transcriptional regulator family (such as the homeobox genes) on the basis of sequence homology.

b) Establishment of "prototype therapeutic" antisense oligonucleotide from a set of test antisense oligonucleotides. These prototype

compounds will be used in the reduction to practice.

i) Synthesize test antisense oligonucleotides using standard procedures, for example, those for producing phosphorothioates (Vu et al, Tetrahedron Lett, <u>32</u>, 3005, 1991).

ii) Using assays for transcriptional regulators or their direct modifiers select prototype therapeutic antisense oligonucleotides out of the set of test compounds on the basis of shutting down expression of the target gene in the cell types to be therapeutically manipulated. In practice, the same set of prototype agents capable of shutting down target gene expression in a variety of cell types could be used in the Reduction to Practice, Step 2, hereinafter, for multiple therapeutic objectives.

b) Synthetic double-stranded oligonucleotides that are ligands for the DNA binding domain of one or more transcriptional regulators. (Wu et al, Gene, <u>89</u>, 203, 1990)

Prototype therapeutic agents of this type for use in the reduction to practice will correspond to actual gene sequences to which the transcriptional regulator(s) will have been

shown to bind using standard techniques such as the gel mobility shift assay. (Ausubel et al (eds.) Current Protocols in Molecular Biology, John Wiley NY, 1989 (updated semiannually).)

5   c)   Expression vectors

In the preferred embodiment a recombinant viral vector will be used (Miller and Rosman, Biotech, 7, 980, 1989) that carries the complete coding sequence of the transcriptional regulator or its
10   direct modifier. This will provide for expression of the regulator or modifier in the cells of interest. It will be constructed and tested using standard methods. (Ausubel et al, supra) Alternatively, the viral vector will
15   carry a sufficiently long antisense sequence to such a regulator or modifier to provide for the blocking of expression of the target gene in the cells of interest.

3) Preparation of Tissue

20   The preferred tissue is primary explant or early passaged. It will be acquired using standard surgical procedures. Tissue processing for culture and/or heterotransplant will be according to established methods. Culture
25   conditions for the disordered cells from the various aberrant program diseases or their normal counterparts are referenced in Table III. These references also provide information on acquiring and processing the appropriate cells.

30   Uses to provide the source material for:

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a) determining the subset of the known transcriptional regulators or their direct modifiers that are expressed by a particular cell type.

b) practicing the collateral inventions; that is, diagnosis and staging an aberrant program disease or for selecting optimal treatment in clinical practice.

c) evaluating possible adverse effects of treatments for aberrant program diseases on cultures of the three major constitutively self-renewing tissues (bone marrow, gastrointestinal epithelium, and skin). These cultures will also be used in some of the reductions to practice involving therapeutic manipulations of normal tissue. Culture conditions, Table IV.

d) The other cultures and heterotransplants to be used in the reduction to practice.

4) Discrimination of normal vs malignant cells in a mixed population.

Standard in situ hybridization procedures for detecting chromosome and/or translocation specific changes will be utilized. (Trask Trends in Genet. 7, 149, 1991).

5) Establish assays for scoring effects of manipulating transcriptional regulator function or their direct modifiers on cellular programming.

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a)   Aberrant program disease tissue -

By definition the affected cells in these
disorders express abnormal patterns of gene
expression that produce the characteristic
clinicopathologic features. Both of these can
be monitored using established molecular and
cellular techniques. The specific parameters to
be assayed for each of the types of aberrant
program disease given as examples are shown in
Table III.

b)   Normal tissue -

Reprogramming normal cell behavior where the
relevant   programs   are   differentiation,
proliferation and viability could serve a
variety of therapeutic uses. These would
include but not be limited to certain _in vitro_
and systemic treatments: (1) expansion of
normal cell numbers _in vitro_ prior to
transplantation; (2) promotion of the growth of
gastrointestinal cells in the treatment of
peptic ulcers and inflammatory bowel disease;
(3) liver regeneration, for example, following
partial destruction by a virus or toxic
chemicals; (4) expansion of one or more
hematopoietic cell lineages for a variety of
clinical purposes including reconstitution of
immune function in immunodeficiencies,
counteracting the effects of agents toxic to
bone marrow and in fighting infection.

All of these changes in normal cellular programming can be readily assessed using established techniques.

B) Reduction to Practice

5   Step 1) Determine the subset of transcriptional regulators, and their direct modifiers, expressed by the aberrantly programmed tissue, the corresponding normal tissue, and the
10   constitutively self-renewing normal tissue. Alternatively make a similar determination for any other normal tissue that is to be therapeutically manipulated in accordance with this
15   invention.

       Step 2) Add or subtract expression of transcriptional regulator(s) or their direct modifiers from cells to be therapeutically reprogrammed and the
20   appropriate control tissue, as previously specified.

               a)   Addition - Use expression vector to insert expressible gene for a particular transcriptional regulator or a direct modifier of a transcriptional regulator into aberrantly
25   programmed cells. The inserted gene will be one that is expressed by the corresponding normal cells, but not by the aberrantly programmed cells.

30   b)   Subtraction -

  i) can be achieved by the use of
      antisense oligonucleotides directed
      to the RNA of a particular
      transcriptional regulator or direct
      modulator or double-stranded
      oligonucleotide ligands for DNA
      binding domain of one or more
      transcriptional regulators

Using prototype antisense oligonucleotide(s) or
double-stranded oligonucleotides block function
of specific transcriptional regulator(s) in
aberrantly programmed cells or normal cells to
be therapeutically manipulated through
reprogramming. Alternatively use an antisense
oligonucleotide directed to a direct modifier of
a transcriptional regulator.

  ii) Using expression vector carrying
      antisense DNA directed to a
      particular transcriptional regulator
      or a direct modifier of a
      transcriptional regulator, install
      the new gene in aberrantly programmed
      cells. The therapeutic effect will
      be determined in advance through the
      use of an antisense oligonucleotide.

Step 3) REPROGRAMMING TEST:

Using the methods and procedures described in
the "Hardware for Reduction to Practice" and
using the information given in Tables III and
IV, perform the following functions.

a) Utilize appropriate culture conditions for normal cells to be therapeutically reprogrammed' or for AP disease, the AP cells plus the corresponding normal cells and constitutively self-renewing normal tissues (gastrointestinal, bone marrow, skin);

b) For AP disease, assay one or more pathogenic features of AP cells such as those shown in Table III, according to established procedures;

c) Treat cultures with prototype agent with reprogramming potential (as oligonucleotides to TR, as oligonucleotide ligands for TR, or expression vectors).

d) Score changes in programming and choose those agents that are therapeutically useful; for example:

1) cancer, myelodysplasiac and myeloproliferative s y n d r o m e a n d atherosclerosis - kill AP cells;

2) AIDS, regenerate CD4$^+$ lymphocytes;

3) Expand normal
hematopoietic stem cells
for bone marrow transplant.

Step 4) Test effect of addition or
subtraction of the function of
particular transcriptional regulators
using the agents selected in an
animal model system if the
therapeutic agents are for systemic
use.

Because of the need for a high degree of target
homology with the corresponding human
transcriptional regulator or its direct
modulator the animals will of necessity nearly
always be non-human primates.

In the case of evaluating agents for the
treatment of aberrant program diseases the
animal may either be afflicted with the disease
and both the efficacy of the treatment and the
side effect documented or the animal may be
normal and only the side effects tested.

Step 5) Any undesirable side effects that
might be produced by the potential
therapeutic agents can be reduced or
eliminated in several possible ways,
all of which can be implemented using
existing technology.

a) Antisense oligonucleotides

Figure I demonstrates that there are cell type specific differences in effects of particular antisense oligonucleotides targeted to different sites on specific RNA transcripts on cell behavior. Such differences can be used to select antisense oligonucleotides that produce the desired therapeutic effects with minimal undesirable side effects.

b)   Double-stranded oligonucleotide ligands

Typically more than one transcriptional regulator can bind to the same double-stranded DNA sequence, but with variable affinities. It is, therefore, possible to change the competitive inhibitor effect of such an agent relative to the potential set of target transcriptional regulators by introducing base changes. These can include mismatches. The melting temperature of the two resulting strands, however, must be $\geq$ 40°C under physiologic conditions. The effect of such changes, therefore, can produce a more favorable therapeutic index.

c)   Expression vectors

The levels of expression and efficiency of gene transfer can be readily adjusted on a tissue specific basis by changes in the viral envelope and/or the promoter/ enhancer combination used to achieve gene expression.

Demonstration of the Reduction to Practice with a P53 Target

Step 1 -

5   It is known that p53 is expressed by primary human leukemia blast cells using the metabolic labeling technique (Smith, et al., J. Exp. Med. 164, 751, 1986.)

Step 2 -

A set of four different phosphorothioate antisense oligonucleotides directed to p53 RNA were prepared using

10   an Applied Biosystems, Inc. (ABI) DNA synthesizer (Model 380B) according to the manufacturer's protocols. An antisense oligonucleotide against the HIV rev gene was used as a negative control. The sequences are set forth in the Sequence Listing hereinafter as SEQ ID NOS:1-4.

15   These were used to treat primary human leukemic blasts, normal human bone marrow, normal human circulating T-lymphocytes, normal adult human gastrointestinal epithelium, normal human fetal gastrointestinal epithelium and Rhesus monkey T-lymphocytes. Destruction

20   of p53 RNA by the antisense p53 oligonucleotides was documented using PCR and/or dot blotting.

Step 3 -

The following effects of the antisense p53 oligonucleotides on cellular programming were evident

25   from the results found.

1)   They can irreversibly block the proliferation of, block stem cell self-renewal, or kill human cancer cells. This coupled with the lack of

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toxic effects on normal tissue indicates these
agents can have a role in the treatment of
cancer. (See Tables V-VII).

2) They promote the proliferation of
gastrointestinal epithelium, indicating a role
in the treatment of peptic ulcer and
inflammatory bowel disease (FIGURE I). The
suppressive effect of these agents on mature
lymphocyte (Table IX) proliferative also
supports their role in diseases such as
inflammatory bowel disease that have an
autoimmune component.

3) The data also demonstrates that there are cell
type specific differences in responses to
antisense oligonucleotides targeted to different
sites on RNA transcripts of the same gene
(FIGURE I). This provides a basis for
optimizing therapeutic effects and for
minimizing undesirable side effects.

4) These results support the general principle that
antisense oligonucleotides directed to a
transcriptional regulator can be used to expand
particular normal adult or fetal tissues in
vitro that could then be used for various
medical purposes including transplantation
(FIGURE I).

5) The cell type dependency of the effects of
particular antisense oligonucleotides directed
to a transcriptional regulator support the
cellular program model in general and the
aberrant program model in particular.

Step 4 -

The ability of the antisense p53 oligonucleotides to
recognize the p53 RNA of Rhesus monkeys was demonstrated
by showing a similar inhibitory effect on mature T-cell
5   proliferation for both Rhesus and human cells (Table
IX).

Two Rhesus monkeys weighing 8.9 kg and 6.8 kg were
infused with 52.5 mg and 75.8 mg of the OL(1)p53
antisense oligonucleotide (SEQ ID NO:4) which was
10   radiolabelled over four hours. In keeping with rodent
data, tissue distribution analysis showed substantial
oligonucleotide uptake compared to the levels needed to
block p53 expression. Excretion studies demonstrated
retention of the infused agent for more than two weeks.
15   During this time and subsequently, the animals were
extensively monitored for signs of toxicity and none
were seen.

Step 5 -

Since no unacceptable side effects were produced in the
20   monkeys, it has not been necessary to modify the
antisense oligonucleotides.

The antisense oligonucleotide selected for practice
of the invention may be any of the types described by
Stein and Cohen, Cancer Research 48:2569-2668 (1988),
25   and including without limitation, unmodified
oligodeoxynucleotides, ethyl- or methyl-phosphonate
modified oligodeoxynucleotides, phosphorothioate
modified oligonucleotides, dithioates, as well as other
oligonucleotide analogs, including those incorporating
30   ribozyme structures, and oligoribonucleotides such as

those described by Inove et al., Nucleic Acids Res.
15:6131 (1987); and Chimeric oligonucleotides that are
composite RNA, DNA analogues (Inove, et al, FEBS Lett.
2115:327 (1987). Oligonucleotides having a lipophilic
5   backbone, for example, methylphosphonate analogs with
ribozyme structures, may prove advantageous in certain
circumstances; these molecules may have a longer
half-life in vivo since the lipophilic structure may
reduce the rate of renal clearance while the ribozyme
10   structure promotes cleavage of the target RNA. Gerlach,
Nature 334:585 (1988).

The oligonucleotides may be formulated into
pharmaceutical compositions and administered using a
15   therapeutic regimen compatible with the particular
formulation. As described further below, with the aid
of present disclosure, those of skill in the
chemotherapeutic arts should be able to derive suitable
dosages and schedules of administration for any of a
20   number of suitable compositions that contain the
compounds. Thus, pharmaceutical compositions within the
scope of the present invention include compositions
where the active ingredient is contained in an effective
amount to kill the cells of the cancer without causing
25   unacceptable toxicity for the patient. However, a
preferred dosage comprises that which is sufficient to
achieve an effective blood concentration of between
about 1 and about 5 micromolar. Although a preferred
range has been described above, determination of the
30   effective amounts for treatment of each type of tumor
may be determined by those of skill in the art of
chemotherapeutic administration.

In addition to the antisense oligonucleotide
35   compounds, the pharmaceutical compositions of the

invention may contain any of a number of suitable excipients and auxiliaries which facilitate processing of the active compounds into preparations that can be used pharmaceutically. Preferably, the preparations will be designed for parental administration. However, compositions designed for oral or rectal administration are also considered to fall within the scope of the present invention. Preferred compositions will comprise from about 0.1 to about 1% by weight of the active ingredients.

Suitable formulations for parental administration include aqueous solutions of the active compounds in water-soluble or water-dispersible form. Alternatively, suspensions of the active compounds may be administered in suitable lipophilic carriers. The formulations may contain substances which increase viscosity, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the formulation may also contain stabilizers. Additionally, the compounds of the present invention may also be administered encapsulated in liposomes. The oligonucleotide, depending upon its solubility, may be present both in the aqueous layer and in the lipidic layer, or in what is generally termed a liposomic suspension. The hydrophobic layer, generally but not exclusively, comprises phospholipids such as lecithin and sphingomyelin, steroids such as cholesterol, more or less ionic surfactants such a diacetylphosphate, stearylamine, or phosphatidic acid, and/or other materials of a hydrophobic nature.

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TABLE I

Analogy with Language

The following analogy with language illustrates the
essential nature of the model of clinical cancer given
5   in the patent application and the basic rationale for
using antisense oligonucleotides directed against the
indicated target or traitor genes as therapeutic agents.
It should be clear that this is a novel, inventive and
useful approach.

10   RULES:

Biology   Language Equivalent

The   instructions   for   a   word
particular   pattern   of   gene
expression (program) where key
15   programs are differentiation,
viability and proliferation

Transcriptional Regulators (or   letters
any of the other types of
regulators listed as target or
20   traitor genes)

Programmed cell death   nonsense letter
                                            combination

Malignant cells have different   malignant cells
program   instructions   than   express unique
25   . corresponding normal cells and   words
normal cells in general

| Normal cells at different stages of differentiation express different program instructions than other cell types | different normal cell types have their own vocabulary |

| All or nearly all the letters used by malignant cells are structurally normal and appear in normal cells | the alphabets of normal and malignant cells are essentially the same |

| As particular programs unfold, the pattern of regulators expressed changes | cells express different words at different program stages |

Note: The words used in the following examples have only a loose correlation to actual cellular behaviors or programs.

Hypothetical Example

	Cell Type 1 (e.g. liver)	Cell Type 2 (e.g. kidney)
Normal	retard	stop
Low grade malignant	start	swarm
High grade malignant	spread	grow

Comments - Table I (cont.)

I. ANALOGY WITH BASIC CLINICOPATHOLOGIC MECHANISMS

    a) "T" and "P" in normal cell type 2 but not in
malignant type 1 could be considered analogous
antioncogenes since they must be deleted for malignant
progression. That is, for the word "stop" to be changed
to the word "swarm". These deletions must occur along
with the deletion of "o" and the addition of "w", "a",
"r" and "m". The same letter "p", however, appears in
the high grade malignant type 1 cell, while "t" appears
in the low grade form. This fits the observations that
antioncogenes are far from universally deleted in human
cancers, that multiple genetic changes appear to be
involved in carcinogenesis and that clinical cancers
typically evolve phenotypically.

    b) "m" and "w" could be considered analogous to
"oncogenes" since they are required for "stop" evolving
to "swarm" and they are not expressed in other normal
adult cells. Alternatively, "m" and "w" could be
normally only expressed at the embryonic-fetal stage of
development.

    c) "s" becomes expressed in the malignant forms of
type 1 cells (ectopic expression) while it is normally
expressed in type 2, but not type 1.

II. ANALOGY WITH ANTISENSE OLIGONUCLEOTIDE
     TREATMENT STRATEGY

    a) Inhibition of "t" expression will kill low
grade type 1 calls but not normal cell types 1 and 2,
because "start" becomes "sar" which is not a word, but

"retard" and "stop" become "read" and "sop" respectively which are both words.

b) Blocking "m" but not "w" will kill malignant cell type 2 at the low grade phase since "swarm" minus "m" becomes "swar" which is not a word; but "swarm" minus "w" becomes "rams", a word.

d) Knocking out "a" will kill low grade 1 and 2 and high grade 1, but it also kills normal cell type 1. So antisense inhibition of "a" might be useful for purging bone marrow of malignant cell type 1 or 2 but not for systemic treatment.

e) Deletion of "r" would not kill normal cell type 1 ("retard" becomes "date"), but it would kill three of the four malignant cell types. The exception being low grade 1 where "start" becomes "sat".

f) Removal of "e" kills normal and high grade type 1, so it would not be expected to be a good target for systemic therapy.

g) Of the remaining letters elimination of "d" or "g" will not result in the death of any of the cell types; removal of "s" will kill high grade 1, but none of the other cell types it appears in; blocking "o" will kill both malignant forms of type 2; and inhibition of "p" will kill high grade 1 but not normal type 2.

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TABLE II

Human Transcriptional Regulators

Members	Family	Where known to be expressed	Representative Reference	Possible Direct Modifications	Reference
p 53	---	proliferating mature T and B lymphocytes, numerous types of cancer	Kern, et al., Sci. 252, 1708, 1991.	phosphorylation	Smith, et al., J. Exp. Med. 164, 751, 1986.
A20	---	endothelial cells	Opipari, et al., J. Biol. Chem. 265, 14705, 1990.	---	---
AF-1	---	liver	Metzger, et al., J. Biol. Chem 265, 9978, 1990.	---	---
AP-2	---	cell lines	Comb and Goodman, Nucl. Acids Res. 18, 3975, 1990.	---	---
AP-4	---	cell lines	Hu, et al., Genes and Dev. 4, 1741, 1990.		
ATF1-8	ATF	cell lines	Hoeffler, et al., Mole. Endocrine 5, 256, 1991.	---	---
C/EBP	---	liver	Friedman and Mukright, Genes and Dev. 4, 1410, 1990.	---	---

TABLE II (CONTINUED)

CHF.10	--	cell lines	Pannuti, et al., Nucl. Acids Res. 16, 4227, 1988.	--	--
CHF.12	--	cell lines	Pannuti, et al., Nucl.AcidsRes. 16, 4227, 1988.	--	--
(PE-RP1	--	fetal tissue, cell lines	Maekawa, et al., EMBOJ. 8, 2023, 1989.	--	--
CPBR	--	cell lines	Muchardt, et al., J. Virol. 64, 4296, 1990.	phosphorylation	Hoeffler, et al., Mol. Endo. 5, 256, 1991.
EJ2	--	cell lines	Nosse, et al., Cell 56, 777, 1989.	--	--
F47	--	cell lines	Nosse, et al., Cell 56, 777, 1989.	--	--
E2A	--	all blasts	Kamps, et al., 60, 535, 1990.	--	--
EBF1	--	cell lines	Clark, et al., Genes Dev. 2, 991, 1988.	--	--
EGR 1-3	EGR	cell lines, PMA induced mononuclear cells	Rangnekar, et al., Nucl. Acids Res. 18, 2749, 1990.	--	--

TABLE II (CONTINUED)

FGR 1,2	ETS	cell lines	Watson, et al., PNAS 85, 7862, 1988.	---
ETS 1,2	ETS	T lymphocytes, fetal liver, astrocytes	Bhat, et al., PNAS 87, 3723, 1990.	---
GLI-1-3	GLI	embryonal carcinoma, myometrium, testis, placenta	Ruppert, et al., Mol. Cell. Biol. 8, 3104, 1988.	---
HKR1-4	HKR	testis, placenta, kidney, colon, lung, brain, embryonal carcinoma	Ruppert, et al., Mol. Cell. Biol. 8, 3104, 1988.	---
HOX 1.4, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 5.1, 5.2, 5.4, 6.1, 6.2, 7	homeobox	cell lines, embryos		
HFPp1-9	HFPp	placenta	Bellefroid, et al., DNA 8, 377, 1989.	---
H-plk	---	placenta	Kato, et al., Mol. Cell. Biol. 10, 4401, 1990.	---
HS1	---	lymphocytes	Kitamura, Nucl. Acids Res. 17, 93167, 1989.	---

TABLE II (CONTINUED)

hMTF	:	lymphocytes	Liou, et al., Sci. 247, 1581, 1990.
IgP	--	placenta	Zabel and Baeuerle, Cell 61, 255, 1990.
ISGF1-3	---	cell lines	Pine, et al., Mol. Cell Biol. 10, 2448, 1990.
JUNB	JUN	cell lines	Nomura, et al., Nucle. Acids Res. 18, 3047, 1990.
c-JUN	JUN	cell lines	Nomura, et al., Nucle. Acids Res. 18, 3047, 1990.
JUN-D	JUN	cell lines	Nomura, et al., Nucle. Acids Res. 18, 3047, 1990.
E8	homeobox	cell lines	Kongsuwan, et al., EMBOJ. 7, 2131, 1988.
lyl-1	--	cell lines	Mellentin, et al., Cell 58, 77, 1989.
MAX	--	cell lines	Blackwood and Eisenman, Sci. 251, 1211, 1991.

TABLE II (CONTINUED)

NFF-1	---	cell lines	Baldwin, et al., Mol. Cell. Biol. 10, 1406, 1990.	---
A-myb	myb	cell lines	Normora, et al., Nucl. Acids Res. 16, 11075, 1988.	---
B-myb	myb	cell lines	Normora, et al., Nucl. Acids Res. 16, 11075, 1988.	---
C-myb	myb	cell lines, hematopoietic tissue	Normora, et al., Nucl. Acids Res. 16, 11075, 1988.	---
C-myc	myc	cell lines, hematopoietic tissue	Gazin, et al., EMBO J. 3, 383, 1984.	---
L-myc	myc	placenta, lung cancer	Kaye, et al., Mol. Cell. Biol. 8, 186, 1988.	---
N-myc	myc	neuroblastoma	Slaman, et al., Sci. 232, 768, 1986.	---
myf5	---	muscle	Braun, et al., Nature 346, 663, 1990.	---

TABLE II (CONTINUED)

			Reference
IIF-E1,2	---	hematopoietic cells	Mignotte, et al., Nucl. Acids Res. 17, 37, 1989.
IIFE6	---	hematopoietic cells	Colin, et al., J. Biol. Chem. 265, 16729, 1990.
IIF-µE1	---	lymphocytes	Sen and Saltimore, Cell 46, 705, 1986.
IIF-µE3	---	lymphocytes	Sen and Saltimore, Cell 46, 705, 1986.
IIFGIIa	---	embryonic tissue, hematopoietic cells	Shannon, et al., Mol. Cell. Biol. 10, 2950, 1990.
IIFGIIb	---	hematopoietic cells	Shannon, et al., Mol. Cell. Biol. 10, 2950, 1990.
IIF-IL6	---	monocytes	Akira, et al., EMBO J. 9, 1897, 1990.
IIF-ₖR	---	lymphocytes, cell lines	Ruben, et al., Sci. 251, 1490, 1991.
IIF-5	---	lymphocytes	Kobr, et al., Mol. Cell.Biol. 10, 965, 1990.
225	---	cell lines, lymphocytes	Wright, et al., Sci. 248, 588, 1990.

TABLE II (CONTINUED)

			Reference		
243	--	lymphocytes	Bours, et al., Nature 348, 76, 1990.	--	--
Oct 1	Oct	cell lines	Johnson, et al., Mol. Cell. Biol. 10, 1982, 1990.	--	--
Oct 2	Oct	cell lines	Johnson, et al., Mol. Cell. Biol. 10, 1982, 1990.	--	--
Oct. 3	Oct	embryonic	Scholer, et al., EMBO J. 9, 2185, 1990 (murine)	--	--
Pit-1	--	pituitary	Chen, et al., Nature 346, 583, 1990.	--	--
FLI	homeobox	cell lines	Shen, et al., PNAS 86, 8536, 1989.	--	--
Fr1	homeobox	cell lines	Kamps, et al., Cell 60, 547, 1990.	--	--
Eb	--	hematopoietic cells, retinal cells	Lee, et al., Sci. 235, 1394, 1987.	phos-phoryla-tion	Yen, et al., Exp. Cell. Res. 192, 289, 1991.
RF-y	--	cell lines	Reith, et al., Cell 53, 897, 1988.	--	--
RF-x	--	lymphocytes	Reith, et al., Genes Dev. 4, 1528, 1990.	--	--

TABLE II (CONTINUED)

Thrombotin	---	cell lines	McGuire, et al., Mol. Cell. Biol. 9, 2124, 1989.	---	---
SCL	---	fetal liver, hematopoietic cells, placenta	Begley, et al., PNAS 86, 10128, 1989.	---	---
Sp-1	---	cell lines	Pugh and Tjian, Cell 61, 1187, 1990.	---	---
SPF	---	cell lines	Norman, et al., Cell 55, 989, 1988.	---	---
Tal	---	cell lines	Chen, et al., EMBOJ. 9, 415, 1990.	---	---
TCF-1	---	lymphocytes	Van de Wetering, et al., EMBOJ. 10, 123, 1991.	---	---
TFE3	---	lymphocytes	Beckmann, et al., Genes Dev. 4, 167, 1990.	---	---
VAu	---	hematopoietic cells	Katzav, et al., EMBOJ. 8, 2283, 1989.	---	---
cfos	fos	hematopoietic cells	Runkel, et al., Mol. Cell. Biol. 11, 1270, 1991.	phosphorylation	Barber and Verma, Mol. Cell. Biol. 7, 2201, 1987.
fos-B	fos	cell lines	Mumberg et al, Genes Dev, 5, 1212, 1991	—	—

TABLE II (CONTINUED)

fra-2	fos		Nishina et al, PNAS, 87, 3614, 1990 (chicken)
glucocorticoid receptor	steroid receptor super family	lymphocytes and numerous other cell types	O'Malley, Mole Endocrin., 4, 363, 1990
androgen receptor	steroid receptor super family	male reproductive organs, muscle	O'Malley, Mole Endocrin., 4, 363, 1990
progesterone receptor	steroid receptor super family	female reproductive organs	O'Malley, Mole Endocrin., 4, 363, 1990
estrogen receptor	steroid receptor super family	female reproductive organs	O'Malley, Mole Endocrin., 4, 363, 1990
estrogen related receptors	steroid receptor super family	female reproductive organs	O'Malley, Mole Endocrin., 4, 363, 1990

TABLE II (CONTINUED)

retinoic acid receptor	steroid receptor super family	hematopoietic cells, epithelial tissue	O'Malley, Mole Endocrin., 4, 363, 1990	—	—
thyroid hormone receptor	steroid receptor super family	numerous tissues	O'Malley, Mole Endocrin., 4, 363, 1990	—	—
vitamin D receptor	steroid receptor super family	hematopoietic and many other cell types	O'Malley, Mole Endocrin., 4, 363, 1990	—	—
mineralocorti-coid receptor	steroid receptor super family	kidney, colon, salivary glands	O'Malley, Mole Endocrin., 4, 363, 1990	—	—
H2F-1	Kruppel zinc finger like family	CHL, placenta	Hromas, et al., J. Biol. Chem. 266, 14183, 1991	—	—
HB9	homeobox	hematopoietic, fetal	Deguchi and Kehrl, Blood 78, 323, 1991	—	—
HB24	homeobox	hematopoietic, fetal	Deguchi and Kehrl, Blood 78, 323, 1991	—	—

TABLE II (CONTINUED)

vHNF1	homeobox	liver	Bach, et al., Nucl. Acids Res. 19, 3553, 1991	—	—
HOX11	homeobox	liver, some T cell leukemias	Hatano, et al., Sci. 253, 79, 1991	—	—
PL2	homeobox	cell lines	Lowney, et al., Nucl. Acids Res. 19, 3443, 1991	—	—
rel		cell lines	Brownell, et al., Mol. Cell Biol. 5, 2826, 1985	—	—
HSF		many cell types	Cunniff, et al., Mol. Cell Biol. 11, 3504, 1991	—	—
NF-AR		cell lines	Won and Baumann, Mol. Cell Biol. 11, 3001, 1991	—	—
CCG1		cell lines	Sekiguchi, et al., Mol. Cell Biol. 11, 3317, 1991	phospho-rylated	Sekiguchi, et al., Mol. Cell. Biol., 11, 3317, 1991
rhom-2	rhom-botin	cell lines, embryonic tissue	Boehm, et al., PNAS 88, 4367, 1991	—	—
rhom-3	rhom-botin	cell lines	Boehm, et al., PNAS 88, 4367, 1991	—	—
GATA-3		T cells	Ho, et al., EMBO J. 10, 1187, 1991	—	—
IF-1		cell lines	Auwerx and Sassone-Corsi, Cell 64, 983, 1991	phospho-rylated	Auwerx and Sassone-Corsi, Cell 64, 983, 1991

TABLE III

Scoring Features of Aberrant Programming
Associated with Pathological Effects

Disease	Cell Type	Representative Culture References	Pathological Features of Aberrant Programming	Pathologic Change Reference
Cancer	A) Hematopoietic	A-1) Eaves, et al., J. Tiss. Cult. Meth. 13, 55, 1991. A-2) Messner, et al., Blood 70, 1425, 1987. A-3) Uckin and Heerema, Leuk. Lymph. 2, 1, 1990. A-4) Callgaris-Capplo, et al., Blood 77, 2688, 1991. A-5) Hoang and McCulloch, Blood 66, 748, 1985.	1) Inappropriate proliferation 2) ability to survive in inappropriate sites in body 3) inappropriately invasive	1-3) Kissane (ed) Anderson's Pathology C. V. Mosby St. Louis, 9th ed. 1990.
	B) Solid Tissue	B-1) Moyer, J. Tiss. Cult. Meth. 8, 63, 1983. B-2) Moyer and Poste (eds.), Colon Cancer Cells, Academic Press, San Diego, CA 1990. (Dr. Moyers' culture system works for most primary human sarcomas and carcinomas)		

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TABLE III (CONTINUED)

Myelo-dysplasia	hematopoietic	a) Firken, et al., Br. J. Haemat. 75, 476, 1990. b) Aoki, et al., Amer. J. Hemat. 35, 6, 1990. c) Nagler, et al., Blood 76, 1299, 1990.	1) impairment of blood cell differentiation as judged by standard clinical diagnostics 2) impaired colony formation by multi-potential progenitors 3) immune abnormalities including (a) deficits in CD4$^+$ lymphocytes and (b) decreased NK cells 4) apoptosis 4c) suppressed clonal expansion of myeloid progenitors from patients but not normals in presence of patient serum	1-3) List, et al., J. Clin. Oncol. 1424, 1990. 4) Clark and Lampert, Leuk. Lymph. 2, 415, 1990. 4c) Donohue, et al., Nature 326, 200, 1982.
Myelo-prolif-erative Disorders	hematopoietic	a) Eaves, et al., J. Tiss. Cult. Meth. 13, 55, 1991. b) Messner, et al., Blood 70, 1425, 1987. c) Fauser and Messner, Blood 58, 1224, 1981.	inappropriate clonal proliferation of particular blood cell lineages	Adamson and Fi-alkon, Br. J. Haemat. 38, 299, 1978.

TABLE III (CONTINUED)

APC/AIDS	hematopoietic	Current protocols in immunology Coligan, et al. (eds.), John Wiley, Inc., N.Y. 1991	1) reduction in CD4$^+$ lymphocytes and an inversion of the CD4$^+$ to CD8$^+$ 2) reduction in CD16$^+$CD8$^+$CD3$^-$ cells 3) functional defects in lymphocytes including: (a) altered responses to certain antigens and mitogens; (b) defect in ability to under-go clonal expansion; (c) abnormalities in IL-2 receptor expression 4) functional defects in other blood cells (a) abnormal TNF production; (b) defective platelet production	1) Fauci, Sci. 239, 617, 1988. 2) Mansour, et al., AIDS Res. Human Retro. 6, 1451, 1990. 3a) Pinching and Nye, Immunol. Today 11, 256, 1990. 3a) Allouche, et al., Clin. Exp. Imm. 81, 200, 1990. 3b) Pantaleo, et al., J. Immunol. 144, 1696, 1990. 3c) Prince, et al., Clin. Exp. Immun. 67, 59, 1987. 4a) Oteh, J. Int. Med. 228, 549, 1990. 4b) Zucker-Franklin and Cao, PNAS 86, 5595, 1989.

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TABLE III (CONTINUED)

| Atherosclerosis | smooth muscle | a) Orekhov, et al., Atherosclerosis 60, 101, 1986.

b) Campbell and Campbell, Vascular Smooth Muscle in Culture, Vols. I and II, CRC Press, Boca Raton, FL, 1987. | Shift from contractile to synthetic phenotype. Features of latter include
1) proliferation
2) increased HLA-DR expression
3) loss of muscle proteins
4) growth factor production
5) synthesis of extracellular matrix
6) production of decay-accelerating factor
7) shift from media to intimal location | 1) Olsson (ed) Atherosclerosis: Biology and Clinical Science, Churchill Livingstone, NY 1987.
2) Jonasson, et al., J. Clin. Invest. 76, 125, 1985.
3) Glukhova, et al., PNAS 85, 9542, 1988.
4) Wilcox, et al., J. Clin. Invest. 82, 1134, 1988.
5) Mosse, et al., Lab. Invest. 53, 556, 1985.
6) Seifert and Hansson, J. Clin. Invest. 84, 597, 1989.
7) Betz, et al., J. Cell. Phys. 147, 385, 1991. |

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TABLE IV

Representative Tissue Culture References
for Primary Normal Human Tissue

Tissue	Reference
Gasterointestinal (and a variety of other epithelial and mesenchymal cell types)	a) Moyer and Gendelman, J. Leuk. Biol. 49, 499, 1991. b) Moyer, J. Tiss. Cult. Meth. 13, 107, 1991.
bone marrow	Eaves, et al., J. Tissue Cult. Meth. 13, 55, 1991.
hematopoietic stem cells	a) Messner, et al., Blood, 70, 1425, 1987. b) Bernstein, et al., Blood 77, 2316, 1991. c) Caux, et al., Blood 75, 2292, 1990.
liver	Gomez-Lechan, et al., In Vitro Cell. Dev. Biol. 26, 67, 1990.

TABLE V

Effect of p53 a.s. ODNs on _in vitro_ growth of partially purified blasts from peripheral blood of patients with acute non-lymphocytic leukemia. Values represent triplicate cultures from seven separate experiments, a through g, including six different patients at either presentation or relapse. Peripheral blood leukemia blasts were isolated by Ficoll-Hypaque separation and sheep erythrocyte T-cell rosetting. Cells were plated at 5×10^5/ml in medium as described (±). Control cultures either contained no a.s. ODNs (control), or a.s. ODN to rev (HIV). A.s. ODNs were added 24 hours after plating. In A., aliquots were removed from culture on days 5, 10 and 15 and counted for Trypan blue exclusion. In B., cells were removed on day 10 washed to remove a.s. ODN, replated at 5×10^5/ml and counted 5 days later (day 15). nd= not done.

A.   percent viable cells of control

a.s. ODN	day	5	10	15		5	10	15
			a				b	
Control		100	100	100		100	100	100
HIV		nd	nd	nd		54	55	75
OL(1)		88	4	1		0	0	0
λ(1)		51	74	nd		0	0	0
λ(3)		101	15	13		51	18	21
C(1)		60	57	41		nd	nd	nd

	5	10	15		5	10	15
		c				d	
	100	100	nd		100	100	100
	46	45	nd		100	79	89
	22	4	nd		64	21	27
	86	21	nd		64	43	29
	30	2	nd		41	24	24
	49	15	nd		55	19	17

TABLE V (CONTINUED)

B.   percent of control

a.s. ODN		e Replated			f Replated			g Replated	
day	5	10	15	5	10	15	5	10	15
Control	100	100	100	100	100	100	100	100	100
HIV	102	85	103	83	103	92	90	97	91
OL(1)	68	48	33	45	44	31	45	34	43
A(1)	77	46	37	53	44	71	59	75	64
A(3)	93	55	37	51	57	73	63	51	62
C(1)	72	49	41	53	59	37	66	63	77

*   Buick, et al., Blood 54, 95, 1979.

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TABLE VI

Effect of p53 a.s. ODNs on _in vitro_ colony formation (CFU-L) of cells removed on either day 0 or 7 of the two of the cultures described in Table V (f and g). Values represent mean ± SD of triplicate cultures. Controls were as described in Table V. Cells were cultured according to B. Lange (*) at 1 x10⁵/ml. A fraction of the cells from day 7 colonies were washed and replated at 1 x10⁵/ml as described (*) in the absence of a.s. ODN. A colony was defined as >20 cells; day 7 control colonies varied from 70 to 240, day 14 colonies varied from 13 to 55. n.d. = not done.

a.s. ODN		f replated		g replated	
	day	7	14	7	14
Control		100	100	100	100
HIV		87	92	98	82
OL(1)		17	15	60	2
A(1)		28	138	79	262
A(3)		58	108	96	24
C(1)		23	8	81	4

*   Lange, et al., Blood 70, 192, 1982.

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TABLE VII

Effect of p53 a.s. ODN on <u>in vitro</u> growth of normal bone
marrow. Values represent the cumulative mean ± SD of
triplicate cultures from three separate experiments.
Mononuclear cells were isolated by Ficoll-Hypaque
separation. Cells were plated at 2 x 10^6/ml in medium as
described (*) except for substituting horse for human
serum. Control cultures either contained no a.s. ODN, or
a.s. ODN to rev (HIV). A.s. ODNs were added 24 hours after
plating. Aliquots were removed from culture on days 5 and
10, and counted for Trypan blue exclusion.

	Viable cells x 10^5	
a.s. ODN	day 5	10
Control	12.7±3.3	13.1±2.5
HIV	11.8±2.6	11.5±2.2
OL(1)	12.1±2.6	12.0±1.8
A(1)	11.9±2.1	12.2±1.5
A(3)	12.1±2.7	14.5±1.4
C(1)	9.7±2.0	10.9±0.5

*   Bayever, et al., Exp. Cell Rev. <u>179</u>, 168, 1988.

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TABLE VIII

Effect of p53 a.s. ODNs on _in vitro_ colony formation of hematopoietic progenitors removed on day 7 from three of the normal bone marrow cultures described in Table VI. Values represent the cumulative mean ± SD of triplicate cultures. Controls were as described in Table V. Cells were cultured as described (*), except they were plated at 1 x 10^5/ml. A fraction of the cells from day 7 colonies were washed and replated at 5 x 10^4/ml for the CFU-Mix and BFU-E, or 1 x10^5/ml for the CFU-GM as described (*). A colony was defined as >20 cells. All colonies were cultured in the absence of a.s. ODNs.

a.s. ODN	CFU-Mix	BFU-E	CFU-GM
Control	3.9±4.5	4.4±7.2	237.6±100.1
HIV	1.1±0.9	1.0±1.0	329.1±161.9
OL(1)	1.8±1.8	15.8±1.8	278.9±117.9
A(1)	9.5±6.7	11.6±7.8	330.3±123.8
A(3)	1.0±1.0	1.3±1.8	261.3±90.2
C(1)	3.4±4.1	1.0±1.9	254.5±94.9

*   Messner, et al., Blood 70, 1425, 1987.
     Caux, et al., Blood 75, 2292, 1990.

TABLE IX

Method for non-human primate peripheral blood T-cell studies:

1. Heparinized blood was diluted by one third with HBSS, layered over Ficoll-Hypaque and centrifuged at 1600 r.p.m., for 40 minutes at 20°C.

2. Interface mononuclear cells were recovered and washed twice with HBSS, resuspended in RPMI 1640 with 10% FCS to 1×10^6/ml in the presence of PHA (10μg/ml).

3. Cells were incubated at 37°C in 5% CO_2 for 72 to 96 hours.

4. Cells were harvested, washed and replated at 5×10^5/ml in medium consisting of RPMI 1640 with 10% FCS and 10% IL-2.

5. After a 24 hour incubation the a.s. ODN was added to the culture at a 10μM concentration.

6. At 2 to 3 day intervals an aliquot was removed and counted for Trypan blue exclusion.

TABLE IX (cont.)

<u>BMC039</u> - PHA-primed human T-cells   8/14/91

KMH

PHA stimulated → Day 4 wash + place in IL-2 =
"Day 0", "Day 1" add 10μM oligo

	<u>Media</u>	<u>A(1)</u>	<u>Oh(1)</u>	<u>A(3)</u>	<u>C(1)</u>	<u>HIV-2</u>
<u>Day 0</u>	$2x10^5$/ml	$2x10^5$/ml	$2x10^5$/ml	$2x10^5$/ml	$2x10^5$/ml	$2x10^5$/ml
<u>Day 2</u>	$8x10^5$	3.6	5	4.2	4.2	6.8
	8.2	4.4	5.2	5	5	7.4
	<u>7.4</u>	<u>3.8</u>	<u>4 </u>	<u>4 </u>	<u>5.2</u>	<u>8.6</u>
	$7.9x10^5$	$3.9x10^5$	$4.7x10^5$	$4.4x10^5$	$4.8x10^5$	$7.6x10^5$
<u>Day 4</u>	$11.2x10^5$	7.2	5.6	4	9.2	10.6
	11.4	6.6	8	6	8.6	10.4
	<u>10.6</u>	<u>8 </u>	<u>6.2</u>	<u>7.2</u>	<u>7.8</u>	<u>10</u>
	$11.1x10^5$	$7.3x10^5$	$6.6x10^5$	$5.7x10^5$	$8.5x10^5$	$10.3x10^5$
<u>Day 7</u>	$18.2x10^5$	7	8.8	13	9.2	15.4
	19.6	7.4	6.6	10.4	10.8	14.2
	<u>18.8</u>	<u>8.2</u>	<u>9.8</u>	<u>11.2</u>	<u>11.6</u>	<u>14.6</u>
	$18.9x10^5$	$7.5x10^5$	$8.4x10^5$	$11.5x10^5$	$10.5x10^5$	$14.7x10^5$

On "Day 4" cells were removed, washed free of oligo and replated at $2x10^5$/ml.

Replated cells → single cells

→ * (Day 4 = $2x10^5$/each)

	<u>Media</u>	<u>A(1)</u>	<u>Oh(1)</u>	<u>A(3)</u>	<u>C(1)</u>	<u>HIV-2</u>
<u>Day 7</u>	$6.3x10^5$	6.6	6.5	6.1	6.5	6.1
<u>Day 9</u>	$7.1x10^5$	6.8	6.8	6.9	6.9	6.7

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TABLE IX (CONTINUED)

<u>BMC028</u> - 2 samples of monkey PB   8/14/91
                                                           KMH

       PHA prime → onto IL-2, then oligo

          <u>Media</u   <u>A(1)</u>   <u>0h(1)</u>   <u>A(3)</u>   <u>C(1)</u>   <u>HIV-2</u>

<u>Day 0</u> $2x10^5$/ml $2x10^5$ ml $2x10^5$ ml $2x10^5$/ml $2x10^5$/ml $2x10^5$/ml

<u>Day 1</u> 10µM oligo   10µM oligo   10µM oligo   10µM oligo   10µM oligo   10µM oligo

<u>Day 4</u>

<u>Primate A</u>
        $1.0x10^5$ 0.c   0.4   0.33   0.53   1.2
        <u>1.13</u>   <u>0.47</u>   <u>0.47</u>   <u>0.27</u>   <u>0.4</u>   <u>1.0</u>
        $1.07x10^5$ $0.53x10^5$ $0.47x10^5$ $0.3x10^5$ $0.47x10^5$ $1.1x10^5$

<u>Primate B</u>
        $1.93x10^5$ 1.67   0.67   0.73   0.53 $1.6x10^5$

<u>Day 6</u>

<u>Primate A</u>
        $2.0x10^5$ 1.4   1.0   1.33   1.4   3.2
        <u>2.93</u>   <u>1.2</u>   <u>1.33</u>   <u>1.07</u>   <u>1.2</u>   <u>2.67</u>
        $2.47x10^5$ $1.33x10^5$ $1.2x10^5$ $1.2x10^5$ $1.33x10^5$ $2.93x10^5$

<u>Primate B</u>
        $2.2x10^5$ $1.2x10^5$ $0.7x10^5$ $1x10^5$ $0.8x10^5$ $2.3x10^5$

<u>Day 8</u> bacteria in A

<u>Primate B</u>
        $2.9x10^5$ $1x10^5$ $0.4x10^5$ $0.7x10^5$ $1.2x10^5$ $2.4x10^5$

<u>Day 10</u> bacteria emerging in B

* * *

    While the present invention has been described in
conjunction with a preferred embodiment and specific

5 examples, the description is not meant to limit it. One
of ordinary skill, with the aid of the present
disclosure, may be able to effect various changes,
substitutions of equivalents and other alterations to
the methods and compositions set forth. Therefore, the

10 protection granted by Letters Patent should not be
limited except by the language of the claims as set
forth below.

                   "SEQUENCE LISTING"

(1) GENERAL INFORMATION

    (i) APPLICANT: BOARD OF REGENTS OF THE UNIVERSITY
                     OF NEBRASKA

    (ii) TITLE OF INVENTION: Methods and Compositions
                             for Therapeutic Cellular
                             Reprogramming

    (iii) NUMBER OF SEQUENCES: (5)

    (iv) CORRESPONDENCE ADDRESS:
         (A) ADDRESSEE: John P. Floyd, Esq.
         (B) STREET: 200 Roger Webster
         (C) CITY: Williamsburg
         (D) STATE: Virginia
         (E) COUNTRY: U.S.A.
         (F) ZIP: 23187-3609

    (v) COMPUTER READABLE FORM:
         (A) MEDIUM TYPE: floppy disk, 5.25 inch,
                           360Kb Storage
         (B) COMPUTER: IBM-compatible, 486/33
         (C) OPERATING SYSTEM: MS-DOS 5.0
         (D) SOFTWARE: WordPerfect 5.1

    (vi) CURRENT APPLICATION DATA:
         (A) APPLICATION NUMBER: not available
         (B) FILING DATE: not available
         (C) CLASSIFICATION: not available

(vii) PRIOR APPLICATION DATA: none

(viii) ATTORNEY/AGENT INFORMATION:
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          (B) TELEFAX: (804) 220-0930

(2) INFORMATION FOR SEQ ID NO:1

    (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 20 nucleotide bases
          (B) TYPE: nucleic
          (C) STRANDEDNESS: single stranded
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: Other Nucleic Acid
          (A) DESCRIPTION: oligonucleotide

    (iii) HYPOTHETICAL: no
    (iv) ANTI-SENSE: yes

       (xi) SEQUENCE DESCRIPTION:SEQ ID NO:1:
              TCTCTCCGCT TCTTCCTGCC   20

(2) INFORMATION FOR SEQ ID NO:2

  (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 20 nucleotide bases
      (B) TYPE: nucleic
      (C) STRANDEDNESS: single stranded
      (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: Other Nucleic Acid
      (A) DESCRIPTION: oligonucleotide

  (iii) HYPOTHETICAL: no
  (iv) ANTI-SENSE: yes

  (xi) SEQUENCE DESCRIPTION:SEQ ID NO:2:
        ATCTGACTGC GGCTCCTCCA   20

(2) INFORMATION FOR SEQ ID NO:3

  (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 20 nucleotide bases
      (B) TYPE: nucleic
      (C) STRANDEDNESS: single stranded

(D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: Other Nucleic Acid
        (A) DESCRIPTION: oligonucleotide

    (iii) HYPOTHETICAL: no
    (iv) ANTI-SENSE: yes

    (xi) SEQUENCE DESCRIPTION:SEQ ID NO:3:
        GACAGCATCA AATCATCCAT.   20

(2) **INFORMATION FOR SEQ ID NO:4**

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 20 nucleotide bases
        (B) TYPE: nucleic
        (C) STRANDEDNESS: single stranded
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: Other Nucleic Acid
        (A) DESCRIPTION: oligonucleotide

    (iii) HYPOTHETICAL: no
    (iv) ANTI-SENSE: yes

    (xi) SEQUENCE DESCRIPTION:SEQ ID NO:4:
        CCCTGCTCCC CCCTGGCTCC   20

(2) **INFORMATION FOR SEQ ID NO:5**

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 20 nucleotide bases
        (B) TYPE: nucleic
        (C) STRANDEDNESS: single stranded
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: Other Nucleic Acid
        (A) DESCRIPTION: oligonucleotide

    (iii) HYPOTHETICAL: no
    (iv) ANTI-SENSE: yes

    (xi) SEQUENCE DESCRIPTION:SEQ ID NO:5:
        AGTCTTGAGC ACATGGGAGG   20

WHAT IS CLAIMED IS:

1.   A method for treating an individual having an
Aberrant Programming disease comprising administering
to said individual an effective amount of a
composition selected from the group consisting of an
expression vector, a double stranded
oligodeoxynucleotide, and an antisense
oligodeoxynucleotide; said composition capable of
regulating expression of a transcriptional regulator,
said transcriptional regulator being expressed by the
Aberrant Programming cells and further characterized
by exhibiting a therapeutically useful change in said
cell behavior in the Reprogramming Test.

2.   The method of claim 1 wherein said Aberrant
Programming disease is AIDS and said transcriptional
regulator is not encoded by HIV.

3.   The method of claim 1 wherein said Aberrant
Programming disease is cancer and said
transcriptional regulator is a Traitor Gene.

4.   The method of claim 1 wherein said Aberrant
Programming disease is cancer and said
transcriptional regulator excludes oncogenes.

5.   A method for treating an individual having a
clinical disorder comprising administering to said
individual an effective amount of a composition
selected from the group consisting of a double
stranded oligodeoxynucleotide and an antisense
oligodeoxynucleotide; said composition capable of
regulating expression of a transcriptional regulator,
said transcriptional regulator being expressed by

therapeutically relevant cells and further characterized by exhibiting a therapeutically useful change in said cell behavior in the Reprogramming Test.

6.   A method for treating therapeutically relevant cells from an individual having a clinical disorder prior to transplantation of the cells back into the individual comprising the steps of:
  a) obtaining therapeutically relevant cells for the individual and
  b) exposing the therapeutically relevant cells to a reprogramming amount of an oligodeoxynucleotide having a sequence complementary to a sequence of RNA transcribed from a transcriptional regulator regulated gene or double stranded oligodeoxynucleotide ligand of a transcriptional regulator present in the transcriptional regulator cells.

7.   The method of claim 6 wherein the cells are taken from prenatal tissue.

8.   The method of claim 6 wherein the cells are taken from a different donor than the individual under treatment.

9.   A method for diagnosing or staging an Aberrant Programming disease comprising identifying the relevant subset of transcriptional regulators expressed by Aberrant Programming cells from an Aberrant Programming patient.

10.   A method for selecting the most efficacious treatment regimen for an Aberrant Programming disease comprising identifying the relevant subset of

transcriptional regulators expressed by Aberrant
Programming cells from an Aberrant Programming
patient.

11.   A method for selecting the most efficacious
treatment regimen for an Aberrant Programming disease
comprising removing Aberrant Programming cells from
an Aberrant Programming patient and culturing with an
antisense oligodeoxynucleotide to a transcriptional
regulator or a double stranded oligodeoxynucleotide
to the DNA binding domain of the transcriptional
regulator to determine optimal treatment.

12.   A method for the selection of a target for the
treatment of an Aberrant Programming disease
comprising (i) determining the subset of
transcriptional regulators and their direct modifiers
expressed by the aberrantly programmed tissue, the
corresponding normal tissue, or the constitutively
self-renewing normal tissue or, alternatively, making
a similar determination for any other normal tissue
that is to be therapeutically manipulated in
accordance with this invention; (ii) adding or
subtracting expression of transcriptional
regulator(s), or their direct modifiers, from cells
to be therapeutically reprogrammed and the
appropriate control tissue; (iii) scoring effect on
cellular programming and selecting potential
therapeutic agents; (iv) testing effect of addition
or subtraction of the function of particular
transcriptional regulators, using the agents
selected, (in an animal model system if the
therapeutic agents are for systemic use), and (v)
reducing or eliminating any undesirable side effects
that might be produced by the potential therapeutic

agents. This embodiment is described in detail hereinafter.

13.   An oligodeoxynucleotide, having between about 10 and about 30 bases, and containing a sequence selected from the group consisting of:.

```
5'-ATCTGACTGC GGCTCCTCCA-3'
5'-GACAGCATCA AATCATCCAT-3'
5'-CCCTGCTCCC CCCTGGCTCC-3'
5'-AGTCTTGAGC ACATGGGAGG-3'.
```

14.   The oligodeoxynucleotide of claim 13 wherein the melting point is equal to or greater than 40°C.

15.   The method of claims 1, 5, 6, 7, 8, 9, 10, 11, and 12 where the effective dose is between about 1 nanomolar and about 5 micromolar in extra-cellular fluid.
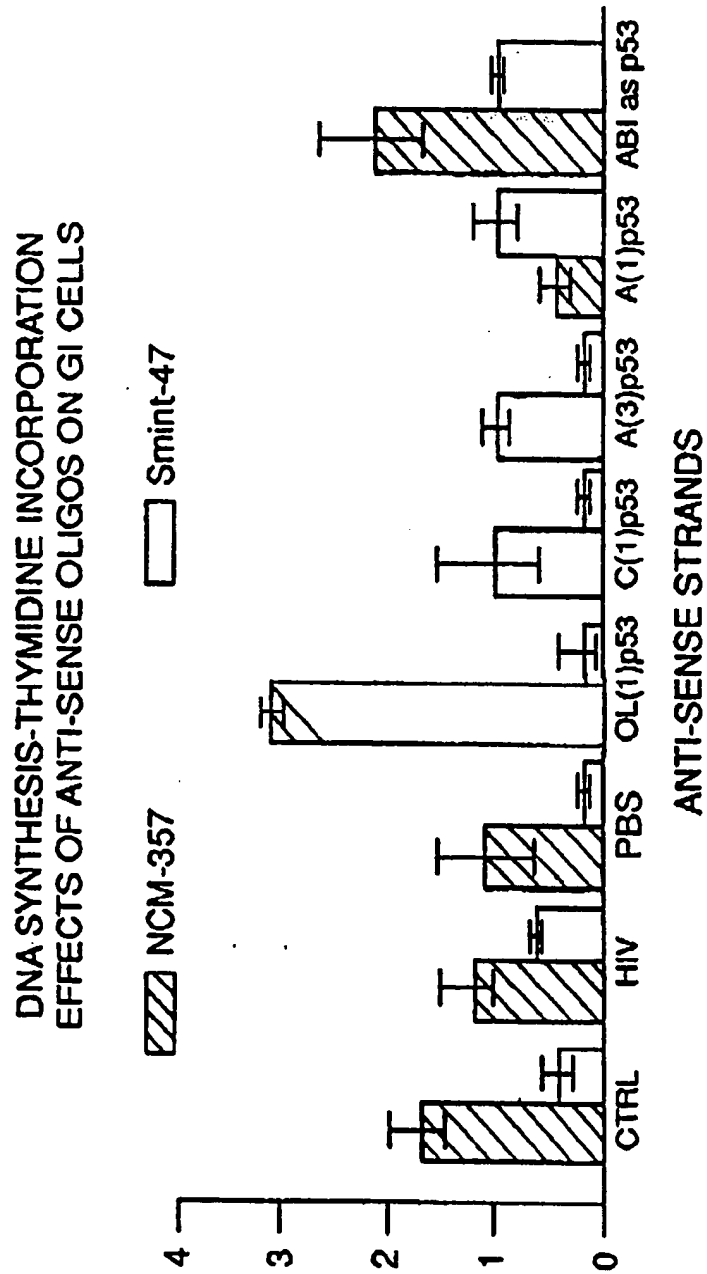
DNA SYNTHESIS-THYMIDINE INCORPORATION
EFFECTS OF ANTI-SENSE OLIGOS ON GI CELLS

FIG. 1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US92/07133

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 48/00
US CL :514/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG: Search terms - combinatorial regulation,   transcriptional regulators, traitor genes, aberrant
programming disease, p53

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Science, volume 250, issued 16 November 1990, BIELINSKA ET AL., "Regulation of Gene Expression with Double Standard Phosphorothioate Oligonucleotides", pages 997-1000, see the entire document.	1-5
Y	Gene, volume 89, issued 1990, W ET AL., "Inhibition of in vitro description by specific double stranded oligodexynucleotides", pages 203-209, see the entire article.	9, 10, 12
P	US, A, 5,087,617 (SMITH) 11 February 1992, see the entire document.	1-15
Y	Journal of Experimental Medicine, volume 164, issued September 1986, SMITH ET AL., "Expression of the p53 oncogene in acute myeloblastic leukemia", pages 751-761, see the entire article.	5
Y	US, A, 4,690,915 (ROSENBERG) 01 September 1987, see the entire document.	6-8, 11

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 OCTOBER 1992	23 NOV 1992

Name and mailing address of the ISA/	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	SUZANNE ZISKA VSH
Facsimile No. NOT APPLICABLE	Telephone No. (703) 308-3964

Form PCT/ISA/210 (second sheet)(July 1992)*.

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EMBO Journal, volume 3, no. 13, issued 1984, MATLASHEWSKI ET AL., "Isolation and characterization of a human p53 cDNA clone: expression of the human p53 gene", pages 3257-3262, see the entire article.	13-15